Purpose: An important measure of articular cartilage function in health and disease is its biomechanical properties. While much research has mouse models of osteoarthritis, the assessment of biomechanical properties in these small joints is quite challenging. We have previously developed novel and easily implemented indentation technique on sheep stifle joints and rat knee joints. The purpose of this study was to determine if this novel technique could be used to map the biomechanical properties of entire mice articular surfaces in indentation.

Methods: Wild-type mice of different age were used for this study. The left and right femoral condyles and tibial plateaus of an 8-week-old C57BL/6 male mouse were first biomechanically mapped in vitro. The mechanical tester used was a 3-axis Mach-1 v500css from Biomomentum Inc. A spherical indenter (Fig. 1) of 0.35 mm diameter (tolerance of ± 2.5 μm and sphericity of 0.625 μm) was used for the indentation measurement with a multiple-axis load cell of 7.0 kg range and 350 mg z-resolution. A 26G intradermal bevel needle was used for the thickness measurement. Following biomechanical testing, the articular surface was fixed in 4% PFA for histological assessment.

Results: The normal force versus time curve (Fig. 2) shows the typical behavior of articular cartilage in a stress relaxation configuration. High-resolution maps of the load at 10μm indentation depth were obtained for the tibial plateau and femoral condyles (Fig. 3). The average load at 10μm of indentation depth (mean ± SD) for the tibial plateau was 5.7 ± 2.7 g and 7.8 ± 4.1 g for the femoral condyles. It takes approximately 1 minute for each indentation measurement and 30 seconds for each thickness measurement (≈25 positions for each condyle and ≈40 positions for each tibial plateau).

Conclusions: The mapping of biomechanical properties of cartilage using indentation is applicable to articular cartilage as thin as mice with any type of articular surface curvature, such as the tibial plateau or femoral condyle. We are currently comparing the thickness found by this novel method with the thickness on histological slides to confirm cartilage thickness maps and cartilage stiffness maps calculated using an elastic model developed by Hayes in 1972. Ongoing studies include examination of the thickness on histological slides with any type of articular surface curvature, such as the tibial plateau or femoral condyle.

Keywords: indentation, cartilage, mechanical properties, articular surface, biomechanical, wedge, mouse, osteoarthritis, cartilage thickness, stiffness maps.
expression differences induced by severe running in mice with data from a human microarray study performed by our group, comparing paired samples of OA cartilage with macroscopically preserved.

**Results:** Among the 1863 significant probes, representing 1699 unique genes, 99 genes showed a differential expression with a fold-change of 1.5 and larger for the equation “running” against “non-running” controls. Of these, 85% (15 up- and 84 down-regulated) of the differential expressed genes were found down-regulated as reaction to mechanical stress. Pathway-analysis of differential expressed genes showed enrichment for the biological processes GO-term: “Skeletal system development”, representing among others: Ctgf, Sox4, Ctnnb1, Mef2c, and Col1a2 (P = 0.0126). Furthermore, the total network was found enriched for protein-protein interactions (22 interactions; P = 0.0024). Figure 1). Of the total 99 genes, a subset of 12 genes was found overlapping in the human and mouse studies (Ctgf, Igfbp5, Bcl6, Egfr, Col1a2, Fbln2, Fam134b, Cdsa, Cdo1, Hspa8, Phospho1 and Rbm5). However, in our human data we found that 8 out of these 12 genes (66%), showed an opposite effect in human cartilage compared to mouse-joints.

**Conclusions:** We found connections between the processes involved in severe mechanical stress in mice and degenerative signs of OA in humans. The fact that we found opposite effects for a majority of these genes needs further investigation. A suggested follow up could be to look at differential expression between load bearing and non-load bearing cartilage of human knee joints.

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**LIPID DISTRIBUTION IN HUMAN KNEE AND HIP ARTICULAR CARTILAGE CORRELATED TO TISSUE SURFACE ROUGHNESS AND SURFACE ACTIVE PHOSPHOLIPID LAYER PRESENCE: EVIDENCE OF COOPERATIVE INTERFACIAL LIPID DELIVERY MECHANISMS**

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**Purpose:** The surface active phospholipid layer (SAPL) of human articular cartilage provides a structural and functional foundation for coordinating surface homeostasis, mitigating certain perturbation events, and generating healing responses. Because damaged articular cartilage lacks SAPL coverage, this feature is used to trait-target damaged tissue for precision resection by physiochemical scalps which mimic azurophilic degranulation of polymorphonuclear neutrophils during the acute phases of wound healing without enzyme system deployment. The resultant wound bed is non-over-resected, phenotype-differentiated, transcriptionally responsive with tissue assembly behaviors, and geographically improved with increased cellular enrichment ratios. Initial evidence from our group indicates that this wound bed is also a suitable substrate for SAPL reconstitution post-treatment through interfacial self-assembly mechanisms. This report presents the in vivo lipid distribution in human knee and hip articular cartilage correlated to tissue surface roughness and SAPL presence reflecting lesion severity.

**Methods:** Under IRB approval, 45 articular cartilage samples were harvested from eight patients (mean age 58.5 ± 13.9) undergoing knee or hip replacement surgery for osteoarthritis. Immediately after harvest, the samples were placed into 4% gluteraldehyde in HEPES buffered saline solution for one hour, micro-sectioned, and stained with Nile red. Laser scanned confocal microscope optical section images (pixel size 460 nm; 235.5 µm squared) were obtained with fluorescence excitation at 532 nm and band-pass filter emission detection centered at 605 nm. Specimens were classified by zonal properties as normal, or deep-, transitional-, or superficial-zone lesions. Lipid distribution was characterized and correlated to lesion surface roughness and SAPL presence. Results: All specimens revealed varying sized lipid droplets limited to the matrix surface region of ~50 µm with droplet size and quantity decreasing significantly as lesion severity increased. Lipid droplets were present within the nuclear envelope and cytoplasm of regional chondrocytes, within the pericellular, lacunar, and extracellular areas, and within the surface extrusion blebs in all groups. Only normal specimens exhibited evidence of SAPL structures with oligolamellar and micellar type configurations. Superficial- and transitional-, but not deep-, zone lesions exhibited small lipid droplet adhesion to the tissue surface in a micellar type configuration with occasional aggregation into lamellar-like structures in areas without significant surface roughness. The following images represent both hip and knee specimens.

**Conclusions:** Lipid distribution in articular cartilage matrix has been described previously as limited to the normal superficial-zone functioning to decrease stiffness and increase compliance via droplet size-dependent nanomechanical influences; however, this surface matrix distribution appears to be maintained at lesion sites as an adaptive response to enhance tissue function commensurate with osteoarthritic chondrocyte phenotype alterations. Because all lesions lacked an observable oligolamellar SAPL, but superficial- and transitional-zone lesions exhibited surface lipid droplet adhesion with micellar type configurations and rudimentary lamellar formation at smooth surfaces, interfacial dysfunction appears to occur prior to the loss of matrix lipid adaptive response capabilities. Because the synovial fluid is pH (proton concentration) responsive and contains over 100 lipid species from...